Summary Report

On

Design, development and evaluation of quercetin and curcumin loaded nanocochleates for the transdermal drug delivery in the treatment of Breast cancer

To be submitted

To

Research Recognition Committee

Of

Ph.D Programme

In

Pharmaceutical Sciences

PhD student

Mrs. Meena Tilawat

M.Pharm

SAP No- 72621017008

Supervisor

Dr Smita Bonde

Associate Professor

Quality assurance

SPTM- Shirpur

SPP School of Pharmacy & Technology Management

SVKM's Narsee Monjee Institute of Management Studies, V.L. Mehta Road,

Vile Parle (W.), Mumbai – 400 056.

Index:

Sr.no	Contents	Page.no
I	Introduction	3
II	Nanocochleates	4
III	Rationale , objectives and plan of work	5-6
IV	Research methodology	6-33
V	Expected outcome and publication details	33
VI	References	34-38

I Introduction:

Breast cancer is the prime cause of mortality in women. The importance of breast cancer as a disease with high incidence and death rate is really high in developing countries. The current available treatment lacks the desired output for the patient. So, the development of efficacious drug delivery which will target only cancerous cells leaving behind normal cells unaffected is the leading requirement. According to global cancer observatory (GLOBOCAN)), it is the most common cancer in women, accounting for 25.1% of all cancers. It was estimated that around 19.7 million cases will occur in next decade, out of that 10.6 million cases will be of less developed countries [1].

The conventional therapy includes chemotherapy, hormone therapy and surgery. But all these therapies fails in complete eradication of cancer cells from body. Hence there is a need of such delivery which target only cancer cells. In the recent years nanotherapy has proven efficient in treatment of breast cancer [2].

Out of various nanocarriers, lipid based drug delivery system extensively applied in many ailments. Being lipid based, these drug delivery facilitates absorption of drug. The various lipid based drug deliveries are divided as emulsions, vesicular systems and lipid particulate systems. Emulsions are divided as nanoemulsions, microemulsions and self-emulsifying drug delivery system etc. Vesicular systems are divided as liposomes, transferosomes, niosomes, ethosomes etc. Lipid particulate system further divided as solid lipid nanoparticles, lipospheres and solid lipid microspheres etc. But all these lipid drug deliveries are having drawbacks such as poor drug loading, stability problem and high aqueous content. Amongst above mentioned dug deliveries, liposomes are extensively applied in many ailments due to improvement in absorption and reduction in adverse effects of drug. They are having the ability to deliver hydrophilic as well as hydrophobic drug moiety. Liposomal drug delivery can be extensively applied to antibacterial, anticancer, immunomodulation, as a diagnostic agent, for vaccine delivery, in genetics etc. Apart from many merits, liposomal drug delivery has many drawbacks such as varying tissue distribution, faster removal of blood and engulfing by reticuloendothelial system (RES) [3-6].

II Nanocochleates:

Nanocochleates are cigar like structures that are formed by the series of lipid bilayers which is generated by the interaction between the negatively charged liposomes and cationic salt usually calcium ions. Phospholipid plays vital role in the formation of nanocochleates as it is the core material in the generation of cochleates. The phospholipids serve as a carrier for the drug**.** Being made up of natural lipids they possess higher stability over the rigorous environmental conditions which is a major concern for the various nanoparticulate system making it more advantageous over others. The various components required for formation of nanocochleates were tabulated in table 1[7-9]

Table 1: Constituents for cochleate assembly

Cationic salt	Zn+2, Mg+2, Ca+2, Ba+2
Phospholipid	Phosphotidyl serine (PS), dioleoylphosphatidylserine (DOPS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidyl glycerol (PG), phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol, diolylphosphatidic acid, distearoyl phosphatidylserine, dipalmitoylphosphatidylglycerol
Category of drug	Protein, peptide, polynucleotide, antiviral agent, anaesthetic agent, anticancer agent, immunosuppressant, steroidal anti-inflammatory agent, nonsteroidal anti-inflammatory agents, tranquilizer, nutritional supplement, vitamin, vasodilator agent, lipoprotein($APOA_1$)

a) Mechanism of action:

It was assumed that the nanocochleates releases the drug when the bilayer of lipid fuses with the cell membrane and the desired pharmacological action may be achieved. Another hypothesis suggests the possibility of phagocytosis involving phosphatidylserine receptors. They are common for lipid membrane of macrophages and outer cochleate membrane. When cochleate approaches closer to lipid membrane of macrophages, it fuses with macrophages, resulting in the release of entrapped drug in the cytoplasm [7-9].

b) Drugs till used for nanocochleates drug delivery:

Amphoterecin B, Tobramycin, Insulin, Rifampicin, Erythromycin, Ketoconazole, Fisetin, Paclitaxel, Raloxifene, Quercetin, Cyclosporin A, Doxorubicin, Docetaxel, Erlotinib HCl, Resveratrol, Curcumin, Hydrocamptothecin, Cytarabine, Proteoliposomes, Artemisinin, Andrographolide, Imatinib etc [9-23].

Rationale:

- Quercetin is an ancient drug used in different ailments
- Despite multiple utilities, it has various drawbacks such as low oral bioavailability, higher dosing, chemical instability, which may lower its efficacy as a therapeutic moiety
- Lipid based drug delivery systems have known to improve its therapeutic efficiency
- Nanocochleates is one of lipid based drug delivery systems extensively studied in recent years for cancer therapy
- By encapsulating quercetin in nanocochleates drug release can be controlled and better efficacy can be obtained as nanocochleates are known to have strong tissue penetration.
- Curcumin and quercetin synergistically inhibit proliferation of cancerous cells by apoptosis [24].

III Objectives and work plan:

The main objective of this research was to design, develop and evaluate quercetin and curcumin loaded nanocochleates for the treatment of breast cancer.

Supporting objectives:

1) To formulate and characterize nanocochleates based drug delivery system.
2) To optimize the formulation using QbD approach.
3) To perform *In vitro, ex vivo* evaluations of developed formulations.
4) To perform stability study as per ICH guidelines

Work plan

Sr.no	Parameters	Time (months
1.	Literature, patent search and review article	03
2.	Procurement of API and excipients	03
3.	Analytical and stability indicating method development and validation	03
4.	Preformulation studies	03
5	Trial batches for placebo nanocochleates, drug loaded nanocochleates and its characerization	02
6.	Process optimization for drug loaded nanocochleates by QbD	02
7.	Development and characterization optimized nanocochleates	04
8.	Evaluation of dosage form	
	▪ *In-vitro* franz diffusion and cell line, *ex-vivo* studies	04
	▪ Stability studies and repeat performance during stability	06
9	▪ Patent and research publication, thesis writing and submission	06
	Total	36

IV Research methodology:

1 Literature search and patent search

Around more than 150 articles and 50 patents were based on nanocochleates drug delivery. In, recent years, from literature search it has been observed that more emphasis was given on cancer research.

2 Procurement of API and excipients:

Curcumin was obtained as a gift sample from Sami labs Bangalore. Quercetin was purchased from Sigma Aldrich Mumbai. Phosphatidylcholine was obtained as a generous gift sample from Lipoid pvt.ltd Germany. Cholesterol was purchased from Molychem Mumbai. Calcium chloride was purchased from Merck Pvt Ltd, Mumbai. All other solvents used were of analytical grade.

3 Preformulation studies:

a) API's: Quercetin:

It is Yellowish amorphous powder. It is insoluble in water, soluble in alcohol, acetic acid. It has melting point-316-318 °C. These all values are matching with standard values given in certificate of analysis [25].

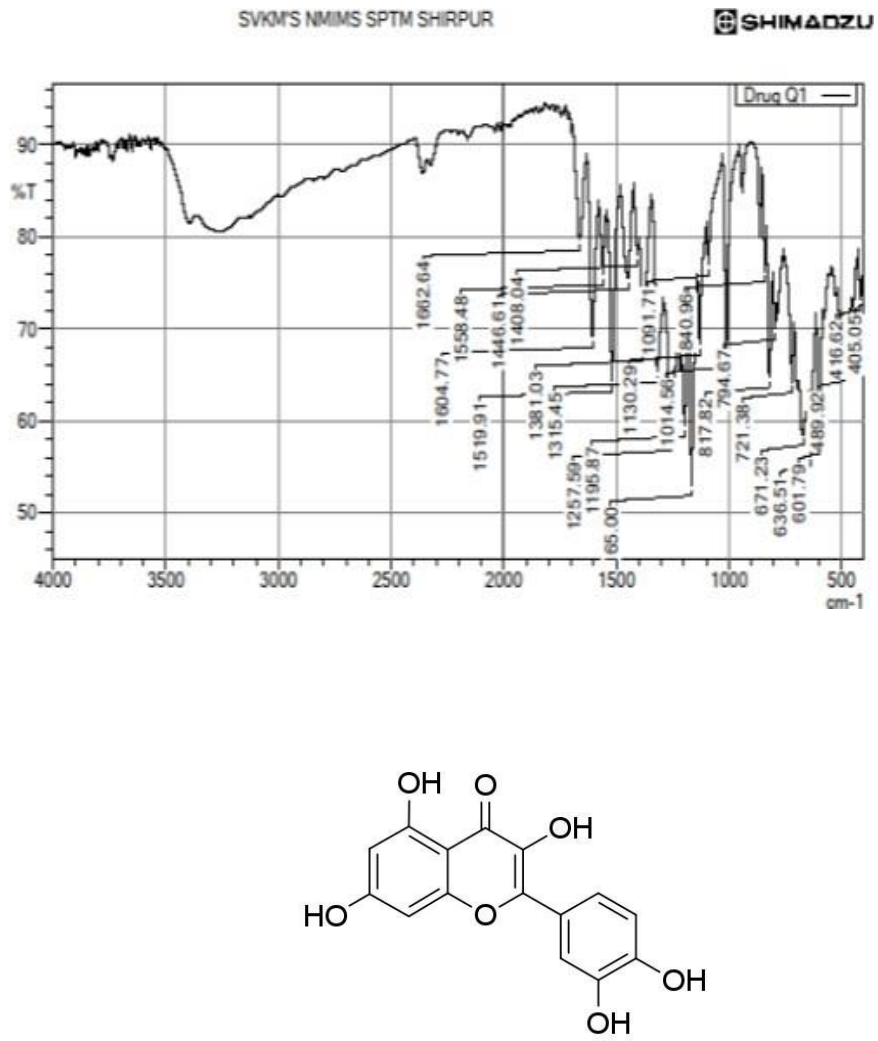


Figure 1: FTIR spectrum and chemical structure of quercetin

Table 2: IR values with functional groups

Functional groups	Standard values	Obtained values
1) OH stretching	3200-3600	3400 broad peak
2) C=O stretching	1670-1820	1662.64
3)C=C stretching(aromatic)	1400-1600	1519.91
4) C-O-C stretching	1050-1100	1130.29

b) Curcumin:

Appearance: Yellowish orange powder

Melting point: 175 -180 °C

Solubility: Insoluble in water, soluble in alcohol [26]

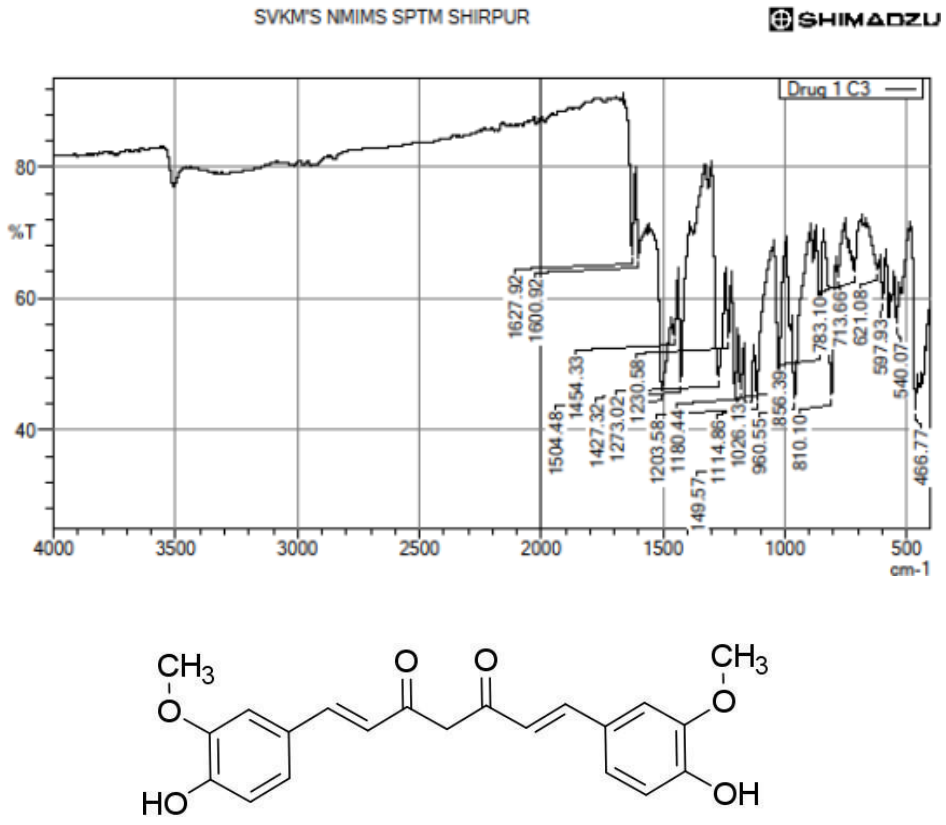


Figure 2: FTIR Spectrum and chemical structure of curcumin

Table 3: IR values with functional groups

Sr.no	Functional groups	Standard values(cm^{-1})	Obtained values
1)	OH- stretching	3200-3600	3500
2)	C=O stretching	1670-1820	1627.92
3)	C=C stretching(aromatic)	1400-1600	1504.48
4)	C-O stretching	1050-1100	1114.86
5)	C=C aliphatic	3000-3200	3050

4 Compatibility of drug and excipients:

Physical mixture of pure drug, excipient individual and combination in 1:1 ratio was kept at different temperature as 4°C, room temperature and elevated temperature 50°C. The samples were kept for 1 month and sample has been withdrawn every 1 week for analysis of functional groups by FTIR studies. After 1 month it has been observed that there is no change in functional groups and only slight fading of colour observed at elevated temperature [27].

5 UV analysis for determination of λmax:

Curcumin 10 mg dissolved in 100 ml of methanol and observed for UV analysis for λmax by UV spectrophotometer.

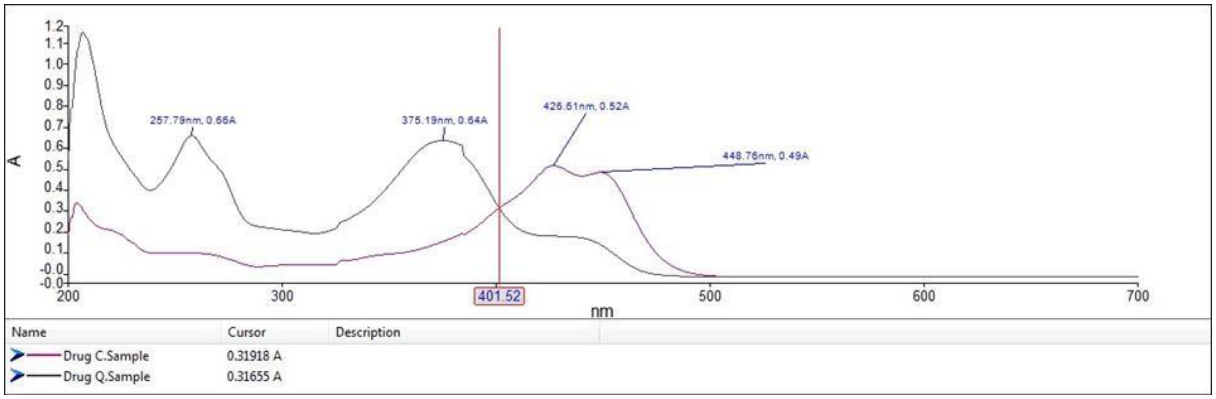


Figure 3: Isobestic point of curcumin and quercetin

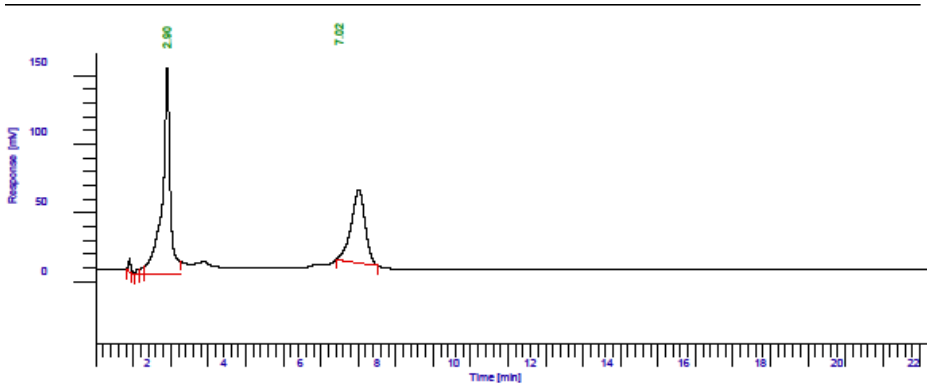
Isobestic point of curcumin and quercetin: Isobestic point of curcumin and quercetin was found to be at 401.52 nm.

6 Analytical method development:

a) High performance liquid chroamatography (HPLC)

Different solvents with varying compositions differ by pH, flow rate and different proportions tried out.

Mobile phase composition containing acetonitrile: methanol: citric acid (5:4:1) at pH 5.4 gives proper resolution of drugs satisfying with system suitability parameters. The chromatograph of simultaneous estimation of quercetin and curcumin was shown in figure 4.



Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		2.903	1883192.05	143706.88	58.84
2		7.018	1369312.65	53076.84	41.33
3					

Figure 4 : Chromatograph of quercetin and curcumin with retention time at 2.90 min and 7.02 min

Sr.no	Parameters	Specifications
1	HPLC system	Perkin Elmer
2	Column	C_{18}
3	Detector	UV visible detector series 200
4	λmax	360 nm
5	Sample volume	20 µL
6	Concentration	10 ppm
7	pH	5.4

Table 5: HPLC method parameters

7. Validation of developed analytical method [28]:

a) Linearity: Different concentrations of curcumin and quercetin were prepared and analysed. Then the concentration against area under curve is plotted and it was found to be linear for both the drugs with R^2 values 0.999 and 0.999 respectively. The linearity for curcumin and quercetin was shown in figure 5 and 6.

Table 7: Concentration with AUC of Curcumin

Concentration (ppm)	Area under curve
1	5451.425
2	9003.415
3	12753.99
4	16851.745
5	21042.7045
6	25126.603

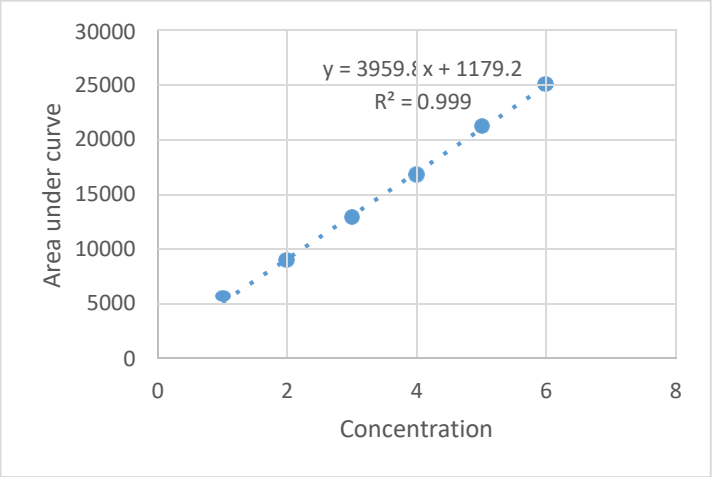


Figure 5: Calibration curve of curcumin

Table 8: Concentration with AUC of quercetin

Concentration (ppm)	Area under curve
1	2546.3
2	4956.3
3	7234.2
4	9434.1
5	12234.2
6	14456.8

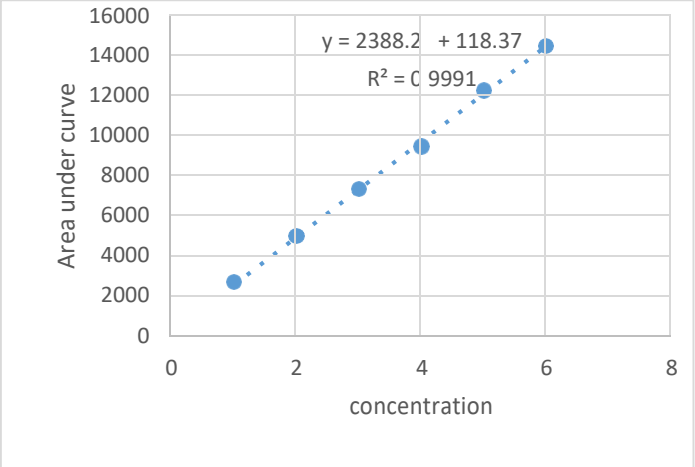


Figure 6: Calibration curve of quercetin

b) Accuracy: Accuracy data showed satisfactory percentage recovery for standard concentration spiked with 40%, 50% and 60% of curcumin and quercetin. Accuracy data was shown in table 9

Table 9: Accuracy data for developed formulation

Initial concentration	Level(%)	Spiked (ppm)	Amount recovered	Recovery(%)	RSD(%)
10	40	4	13.92	99.42.	0.28
10	50	5	15.22	101.46	0.082
10	60	6	16.25	101.56	0.110

c) Robustness: When changing the wavelength, it was found that the % RSD and SEM values were within the 2% (0.034 for quercetin and 0.014 for curcumin) limit, implying that there were no significant changes in method parameters such as retention time, tailing factor, and theoretical plates. Robustness results with change in wavelength were shown in table 10.On changing the flow rate, the % RSD values were found to be 0.030 for quercetin and 0.015 for curcumin respectively As a result, it was discovered that the developed analytical method was robust. Robustness results with change in flow rate were shown in table 11.

Table 10: Robustness results with change in wavelength

Initial concentration (ppm)	Wavelength of detection (nm)	AUC for quercetin	AUC for curcumin
3	358	445865.3	332789.6
3	360	419758.8	342328.16
3	362	420665.3	338568.2
% RSD		0.034	0.014

Table 11: Robustness results with change in flow rate

Initial concentration (ppm)	Flow rate in mL/min	AUC for quercetin	AUC for curcumin
10	0.8	415865.3	326789.6
10	1.2	431658.8	319689.2
10	1.4	443265.3	328568.2
%RSD		0.031	0.014

d) Specificity: Specificity testing was performed, and it was discovered that there is good resolution between the curcumin and quercetin peaks. There was no extra peak observed between these peaks. The chromatograph of blank (mobile phase) and the placebo shows no interference at these retention times, indicating that this method is specific for the intrinsic stability of curcumin and quercetin.

e) Precision: Repeatability was performed by injecting concentration containing 10 ppm of curcumin and quercetin six times under the same optimised conditions and recording the area under the curve. Curcumin and quercetin's relative standard deviations were calculated and found to be 0.012 and 0.004, respectively. As a result, it has been determined that the developed method is precise. Precision results have been shown in table 12.

Table 12: Precision results

Initial concentration ppm	AUC for quercetin	AUC for curcumin
10	1883192.05	1369312.65
10	1825436.2	1356825.23
10	1879582.3	1359823.46
10	1876344.2	1365632.54
10	1879863.2	1354328.23
10	1884523.2	1369875.98
%RSD	0.012	0.004

f) LOD and LOQ: The LOD and LOQ for developed method found to be 0.052 µg/mL and 0.16 µg/mL respectively. These results suggested a high degree of sensitivity for qualitative and quantitative analysis of curcumin and quercetin as API and in formulation.

g) System suitability: System suitability was performed by injecting six replicates of standard concentration of curcumin and quercetin. The data signifies no significant difference in parameters such as retention time, tailing factor, number of theoretical plates and the values of % RSD and SEM was found to be less than 1%. Hence these results showed high degree of accuracy of developed method. Any interference from nanocochleates formulation was not observed.

7 Formulation of quercetin loaded liposomes:

Quercetin liposomes were formulated by the ethanol injection method. The specific amount of phosphatidylcholine, cholesterol, and quercetin was dissolved in 2mL ethanol and heated to the phase transition temperature of lipid (40 °C). This warm solution is rapidly injected in 10mL volume of distilled water kept for constant heating at 40° C and stirring (560 nm) for 30 min.The liposomes generated as soon as the lipid solution come in contact with an aqueous phase. The liposomes were centrifuged at 15,000 rpm, for 30 min at 4 °C. (Cooling microfuge REMI) and washed with distilled water. Centrifugation and washing cycles were repeated twice to confirm the complete removal of the unentrapped drug. The formed liposomes were resuspended in distilled water to make up the volume 10mL and size reduced by probe sonication for 2 min on an ice bath (Ultrasonic probe sonicator VCX-130) filtered through a membrane filter (0.2 mm) and stored at 2–8 °C till further use [29].

8 Fabrication of quercetin loaded nanocochleates:

The liposomes were prepared by using the optimized formula as per the method given in Section Preparation of quercetin loaded liposomes but without centrifugation. The optimized quercetin-loaded liposomes were further converted to nanocochleates using the trapping method by treating with 0.1M solution of calcium chloride [30]. More specifically, to the optimized liposomes, 50 mL of 0.1M solution of calcium chloride was added dropwise under vortex mixing. The turbidity produced by the addition of calcium salt indicates the formation of nanocochleates. Centrifugation and washing cycles were repeated twice to confirm the complete removal of the unentrapped drug. The nanocochleates were resuspended in distilled water and size reduced by probe sonication for 2 min on an ice bath (Ultrasonic probe sonicator VCX-130) filtered through a membrane filter (0.2 mm) The formed nanocochleates were

freeze-dried using 1% mannitol as a cryoprotectant [SP scientific USA (Vertis)]. These nanocochleates were further evaluated for various parameters such as particle size, encapsulation efficiency, and drug release studies.

9 Box-Behnken design: Box Behnken design is a factorial design with three factors at three levels, i.e., low, medium, and high levels [31]. Total 17 experiments were performed, out of which 5 were to check the reproducibility of the experiments. Preliminary studies by preparation of liposomes revealed that amount of lipid, cholesterol, and stirring speed are critical parameters to obtain a desired quality formulation of liposomes. The traditional method of preparation of liposomes is challenging to produce reproducible results; hence, the formulation needs to be optimized. Box Behnken design with three factors and three levels selected for optimization. The dependant and independent variables are given in Table 13.

Table 13: Variables and their levels used in Box-Behnken design

Variables	**Low**	**Medium**	**High**
Independent variables			
A = Amount of Lipid (mg)	40	60	80
B = Amount of cholesterol(mg)	10	15	20
C = Stirring speed(rpm)	300	450	600
Transformed values	-1	0	1
Dependent variables R1= Particle size(nm) R2= Encapsulation efficiency (%) R3= Drug release (%)			

The prediction equations for particle size, encapsulation efficiency and drug release were as follows

Particle size = 358.74 + 33.93A - 47.54B - 39.39C + 39.42AB - 41.48AC - 43.10BC - 24.50A^2 + 30.33B^2 -112.02C^2

Encapsulation efficiency = 76.4 5+ 4.10A + 2.15B + 3.15 C

Drug release = 82.90 - 3.67A + 0.2500 B - 1.78 C+ 0.2750 AB - 0.5250 AC + 0.1750 BC + 0.7125 A^2 + 0.5625 B^2 - 2.54 C^2

The effects of variables on particle size, encapsulation efficiency and drug release were shown by contour plots in figure 7, 8 and 9

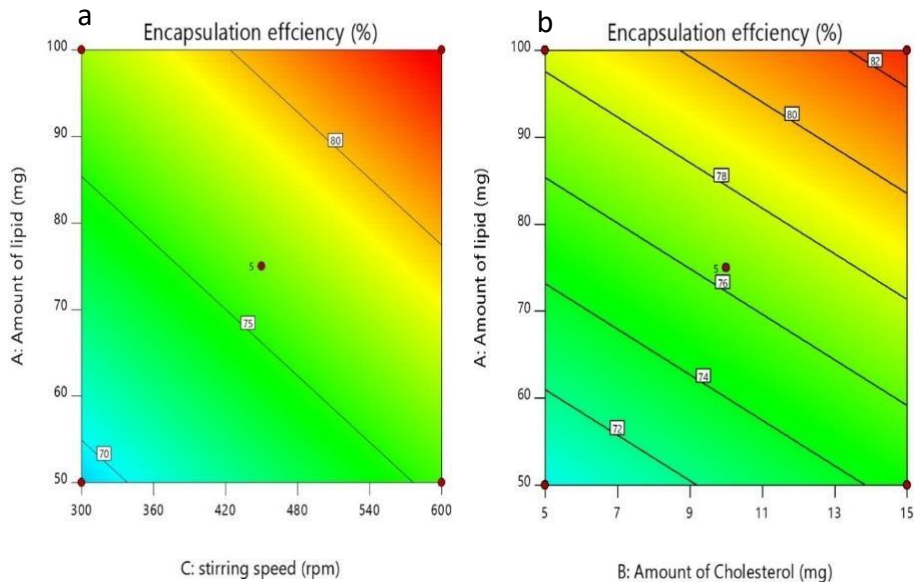


Figure 7: Contour plot showing the effect of independent variables on encapsulation efficiency: A) effect of amount of lipid and stirring speed: B) effect of amount of lipid and amount of cholesterol

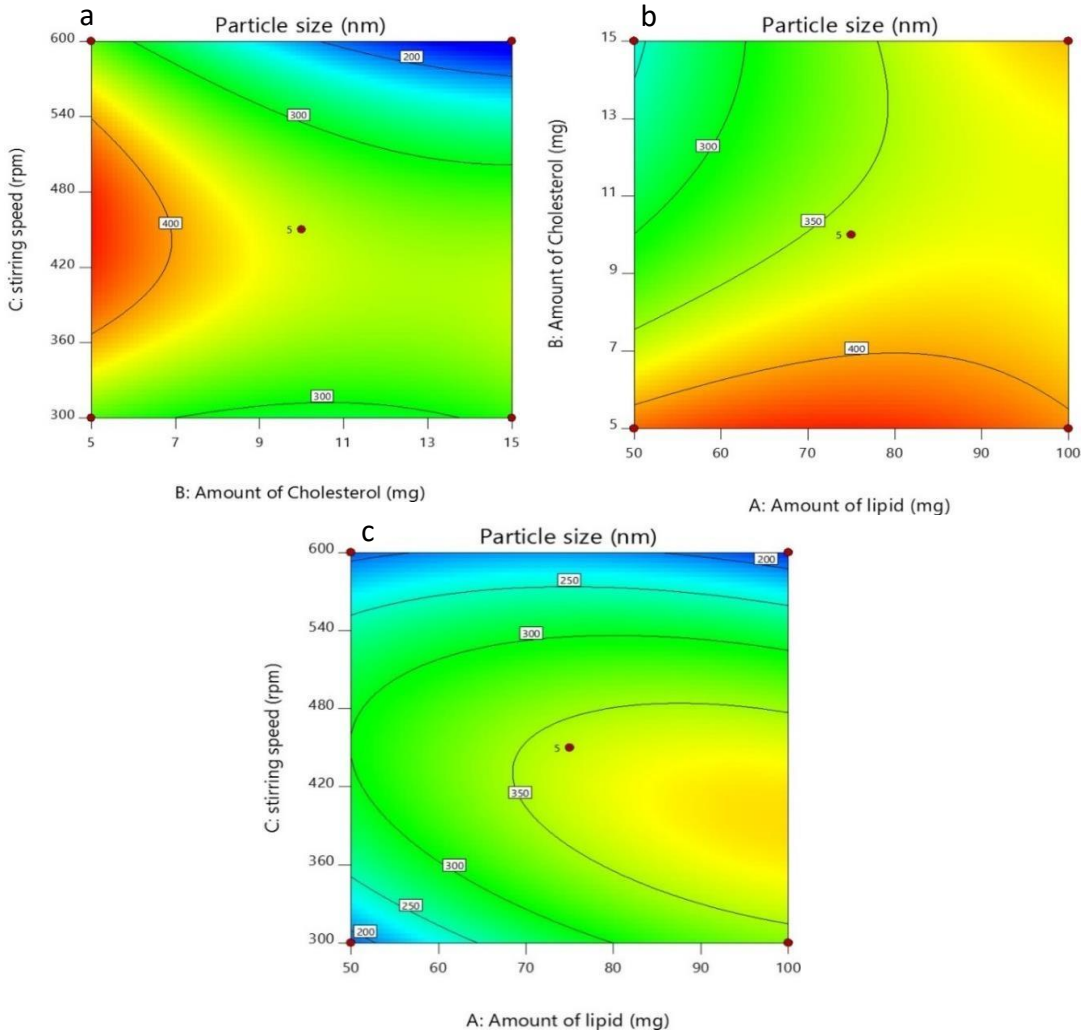
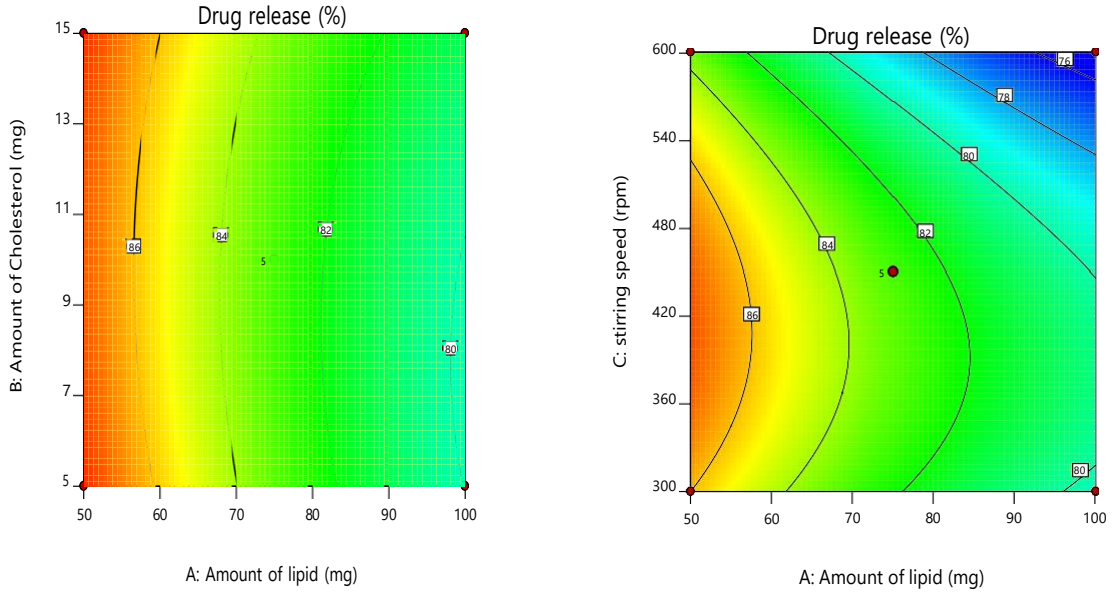


Figure 8: Contour plots showing the effects of independent variables on particle size: A) effect of stirring speed and amount of cholesterol; B) effect of amount of cholesterol and amount of lipid; C) effect of stirring speed and amount of lipid



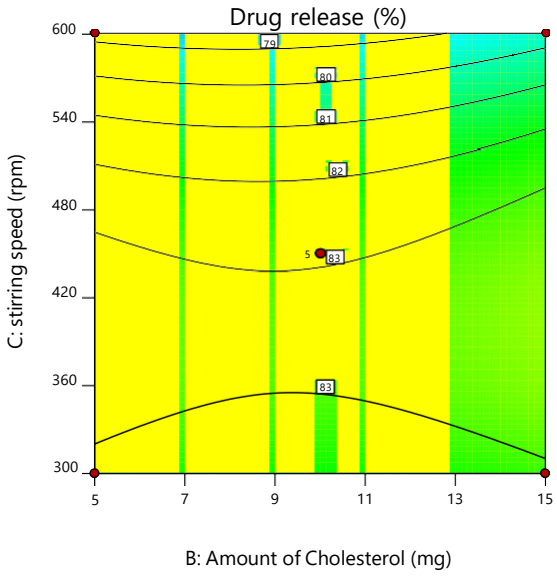


Figure 9: Contour plots showing the effects of independent variables on Drug release A) Amount of lipid and amount of cholesterol B) Amount of lipid and stirring speed C) Amount of cholesterol and stirring speed

Table 14: Predicted and observed variables of quercetin liposomes

Std	Run	A	B	C	Predicted responses			Observed response		
					R1	R2	R3	R1	R2	R3
		mg	mg	rpm	%	nm	%	%	nm	%
5	1	50	10	300	69.2	186.2	85.9	69.3	185.2	85.2
12	2	75	15	600	81.7	147.0	79.5	80.1	160.5	79.3
8	3	100	10	600	83.7	175.2	75.0	83.4	176.3	75.9
3	4	50	15	450	74.5	243.6	87.8	81.4	205.8	88.2
11	5	75	5	600	77.4	328.3	78.7	81.3	289.4	78.3
7	6	50	10	600	75.5	190.3	83.4	65.5	214.8	83.4
2	7	100	5	450	78.4	406.6	79.9	78.4	444.5	79.6
9	8	75	5	300	71.1	320.8	82.6	69	307.4	82.9
10	9	75	15	300	75.4	312	82.7	72	350.9	83.2
13	10	75	10	450	76.4	358.7	82.9	69	370.2	84.8
4	11	100	15	450	82.7	390.3	81.0	83.2	375.9	80.5
15	12	75	10	450	76.4	358.7	82.9	81.6	398.5	83.2
1	13	50	5	450	70.2	417.6	87.8	70.8	432.1	88.4
14	14	75	10	450	76.4	358.7	82.9	81.2	437.7	84.9
6	15	100	10	300	77.4	337.0	79.7	74.8	312.6	79.8
17	16	75	10	450	76.4	358.7	82.9	80	289.3	80.4
16	17	75	10	450	76.4	358.7	82.9	78.6	298	81.2

Optimised batch with 50 mg lipid, 15 mg cholesterol and stirring speed 554.68 rpm showed particle size 160.5 nm, Encapsulation efficiency 76.69% and drug release 85.83%

10 Characterization of quercetin loaded nanocochleates:

a) Particle size, zeta potential and polydispersity index

The liposomal suspension and nanocochleate suspension were diluted 10 times with distilled water and evaluated for particle size, zeta potential and polydispersity index by zetasizer (Malvern, USA). The particle size, zeta potential, polydispersity index of quercetin-loaded liposomes have been found as 160.5±1.970 nm, -27.4mV, and 0.493, respectively. The particle size, zeta potential, and polydispersity index of quercetin-loaded nanocochleates were found to be 327 ±3.681 nm, -16.8mV with polydispersity index 0.396. It is shown in Fig.10

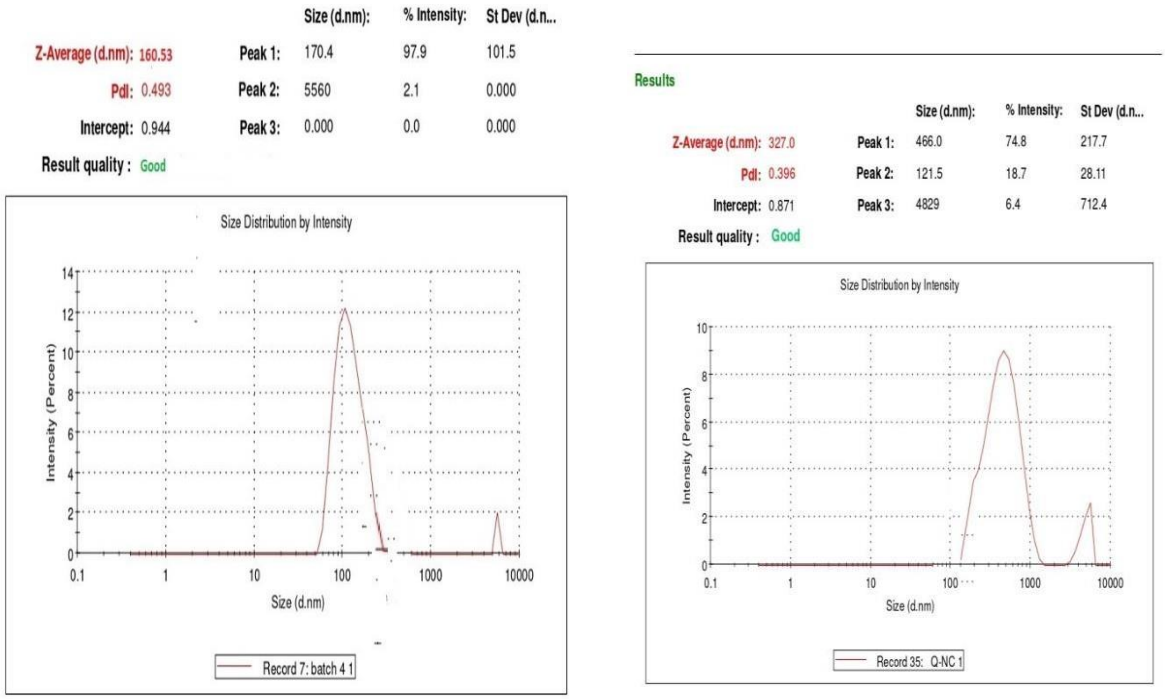

Size (d.nm): % Intensity: St Dev (d.n...
Z-Average (d.nm): 160.53 Peak 1: 170.4 97.9 101.5
PdI: 0.493 Peak 2: 5560 2.1 0.000
Intercept: 0.944 Peak 3: 0.000 0.0 0.000
Result quality : Good
Size Distribution by Intensity
Intensity (Percent)
Size (d.nm)
Record 7: batch 4 1
Results
Size (d.nm): % Intensity: St Dev (d.n...
Z-Average (d.nm): 327.0 Peak 1: 466.0 74.8 217.7
PdI: 0.396 Peak 2: 121.5 18.7 28.11
Intercept: 0.871 Peak 3: 4829 6.4 712.4
Result quality : Good
Size Distribution by Intensity
Intensity (Percent)
Size (d.nm)
Record 35: Q-NC 1

Figure 10: Image showing particle size of quercetin liposomes and quercetin nanocochleates

b) Entrapment efficiency:

2 mL of ethanol was added to 2 mL suspension of liposomes and vortex mixed for 1 min to disrupt liposomes and release quercetin. It was centrifuged (Cooling microfuge REMI) at 15000 rpm for 30 minutes at 4°C. 2 mL of supernatant was removed, diluted by methanol to 5 mL and analyzed at 378 nm by UV spectroscopy [32]. The entrapment efficiency was calculated by equation 1.

50 µl of pH 9.5 EDTA was added to the 2 mL of quercetin nanocochleates to reopen the spiral structure of nanocochleates and release quercetin. 2 ml ethanol was added, vortex mixed for 1 min to disrupt liposomes and release quercetin completely. It was centrifuged (Cooling microfuge REMI) at 15000 rpm for 30 minutes at 4°C. 2 mL of supernatant was removed, diluted by methanol to 5 mL and analyzed at 378 nm by UV spectroscopy. The entrapment efficiency was calculated by equation.

EE = (Amount drug measured/ Amount of drug added) X 100 ---------- (1)

The entrapment efficiency of quercetin-loaded liposomes and nanocochleates was determined was found to be 76.69% ± 0.944, and 83.28 ± 1.745 %, respectively.

c) *In vitro* drug release studies:

The *in vitro* release studies have been performed by using the dialysis bag technique.

In vitro release studies of quercetin-loaded liposomes and nanocochleates were performed in phosphate buffer pH 5.3, which is the pH of cancer cells, and compared with that of free drug. The 1mL of liposomal suspension was placed in a dialysis bag, tied both ends with the thread, and immersed in 100 mL of phosphate buffer. This assembly was kept on a magnetic stirrer at a speed of 100 rpm at temperature37 ± 0.5° C. The 2mL aliquots were withdrawn at specific time intervals (30, 60, 90, 120, 180, 210, 240, 300, 360, 480,600 and up to 1440 min) and the media was replaced with the same amount of buffer [33]. The aliquots were analyzed by UV spectroscopy at 378 nm. The same procedure was followed for drug release studies of quercetin-loaded nanocochleates

It has been found that 90% of the drug is released in about 22 h from quercetin liposomes. The percentage drug release data were subjected to various release models, and it was observed that the data fits Korsmeyer Peppas, drug release model with r^2 value 0.99 and n=0.27. In the case of quercetin-loaded nanocochleates, drug release was delayed further and was found to be 80.12% until 24 h. Percentage drug release profile has been shown in Figure 11. In contrast to it, the 80% drug release from the free drug is observed within 6 h. The release pattern of the drug from quercetin loaded nanocochleates was analyzed for different models, and it showed a significant fit for the Korsmeyer Peppas model (r^2 = 0.99, n=0.15), suggesting that the diffusion mechanism is involved in controlling the percentage drug release from nanocochleates In

comparison to liposomes, the release of drug is further delayed in nanocochleates, which maybe because of the tight junction of a phospholipid, which holds the drug for a longer duration.

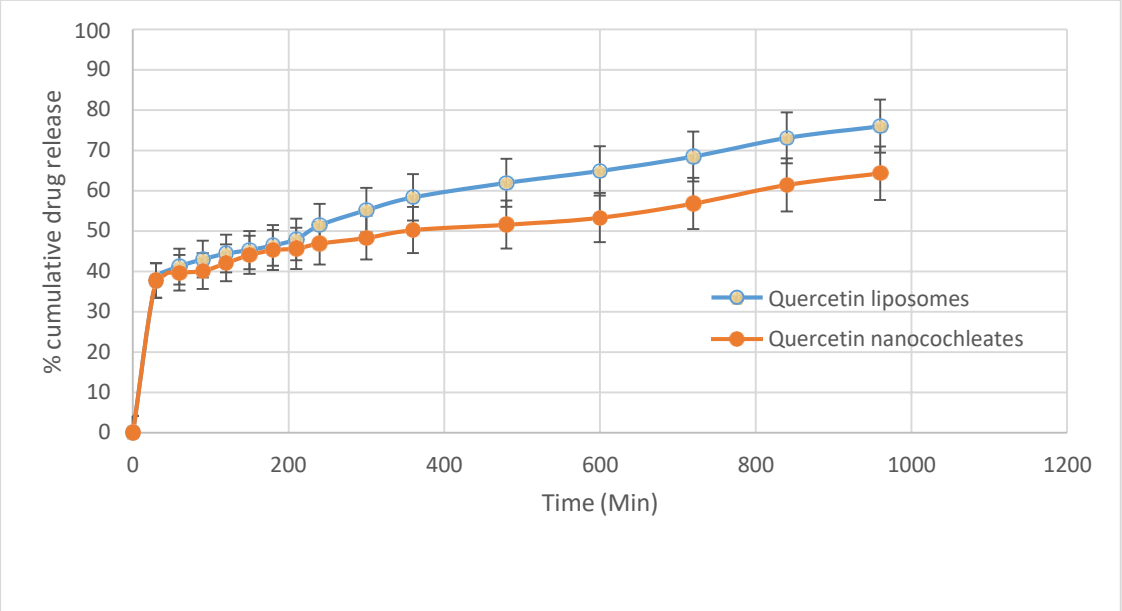


Figure 11: Percentage drug release from quercetin loaded liposomes and quercetin loaded nanocochleates

d) Differential scanning calorimetry:

The pure drug, cholesterol, lipid, quercetin-loaded liposomes, and nanocochleates have been analyzed by differential scanning calorimetry (Mettler Toledo DSC1 Star e system Software Mettler STAR e Default DB v.13) to observe thermal behaviour.

The thermogram of pure drug, cholesterol, phosphatidylcholine, quercetin liposomes, and quercetin-loaded nanocochleates was analyzed by nitrogen flow with a constant increase of heating 10°C/min in a temperature range 0 to 500°C. The thermograph is shown in fig.12. The cholesterol peak is at 150 °C, phosphatidylcholine peak is observed at 230 °C, and a sharp peak of quercetin is observed at 332.8 °C. Further, the peaks of quercetin in quercetin liposomes and quercetin nanocochleates are shifted to lower temperature indicating quercetin is encapsulated in liposomes and nanocochleates respectively [34].

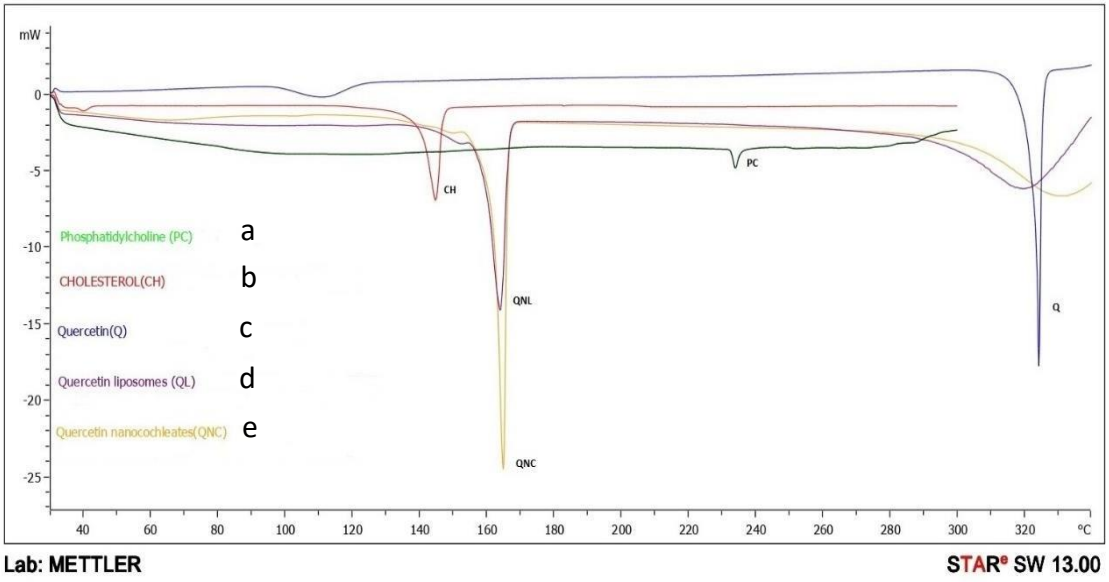


Figure 12: Differential scanning calorimetry of a) phosphatidylcholine, b) cholesterol, c) quercetin, d) quercetin loaded liposomes, and e) quercetin loaded nanocochleates

e)* Surface morphology*:

The scanning electron microscopy reveals external surface characteristics, crystalline nature, and chemical composition of the sample. The surface morphology of quercetin-loaded nanocochleates was observed by field emission SEM as shown in fig.13. The rod shape of nanocochleates is confirmed in SEM image.

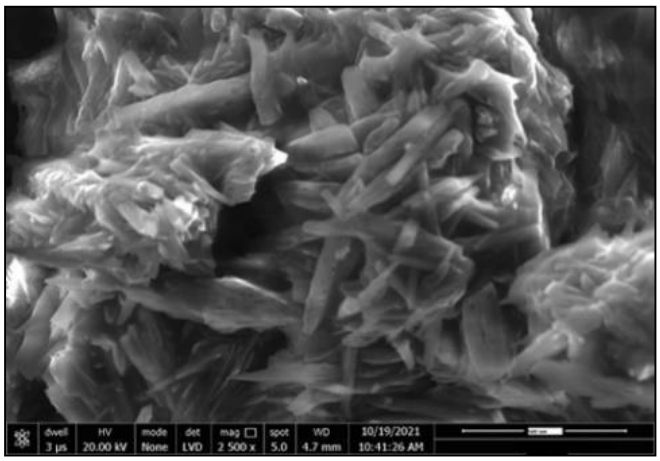


Figure 13: Image Showing Scanning electron microscopy

11 Formulation of curcumin loaded liposomes:

Curcumin loaded liposomes were prepared by ethanol injection method. The specific amount of Phosphatidylcholine, cholesterol and curcumin were dissolved in 2 ml ethanol and heated up to temperature 40°C. This warm solution is injected rapidly in 10 ml distilled water and stirred at specific rpm on magnetic stirrer (Remi, India) for 30 min. Then the formulated vesicles of curcumin and quercetin filtered through 0.45 µm membrane filter to get purified liposomes. The liposomes further sonicated for by probe sonicator for 2 min on ice bath (Ultrasonic probe sonicator VCX-130) [36-37].

12 Formulation of curcumin loaded nanocochleates:

Curcumin loaded nanocochleates have been formulated by adding specific amount calcium chloride (0.1 mM) as bridging agent to curcumin liposomes under vortex. The formation of curcumin loaded nanocochleates confirmed as the liposomal suspension becomes turbid and aggregates of nanocochleates observed as calcium ions comes in contact with liposomes. The formulated nanocochleates further sonicated by using probe sonicator for 2 min on ice bath (Ultrasonic probe sonicator VCX-130) [36-37] .

13 Characterization of curcumin loaded nanocochleates:

a) Determination of particle size:

The particle size of curcumin liposomes and nanocochleates, were determined by using zetacizer (Malvern, USA). Prior to measurement, each sample was diluted with distilled water.

The particle size of curcumin liposomes and curcumin nanocochleates were found to be 235.6 nm and 328.6 nm respectively.

Zeta potential of curcumin liposomes and curcumin nanocochleates were found to be -25.6 and 15.0 nm mV.

Figure 14 reveals particle size, zeta potential and polydispersity index of curcumin liposomes and nanocochleates

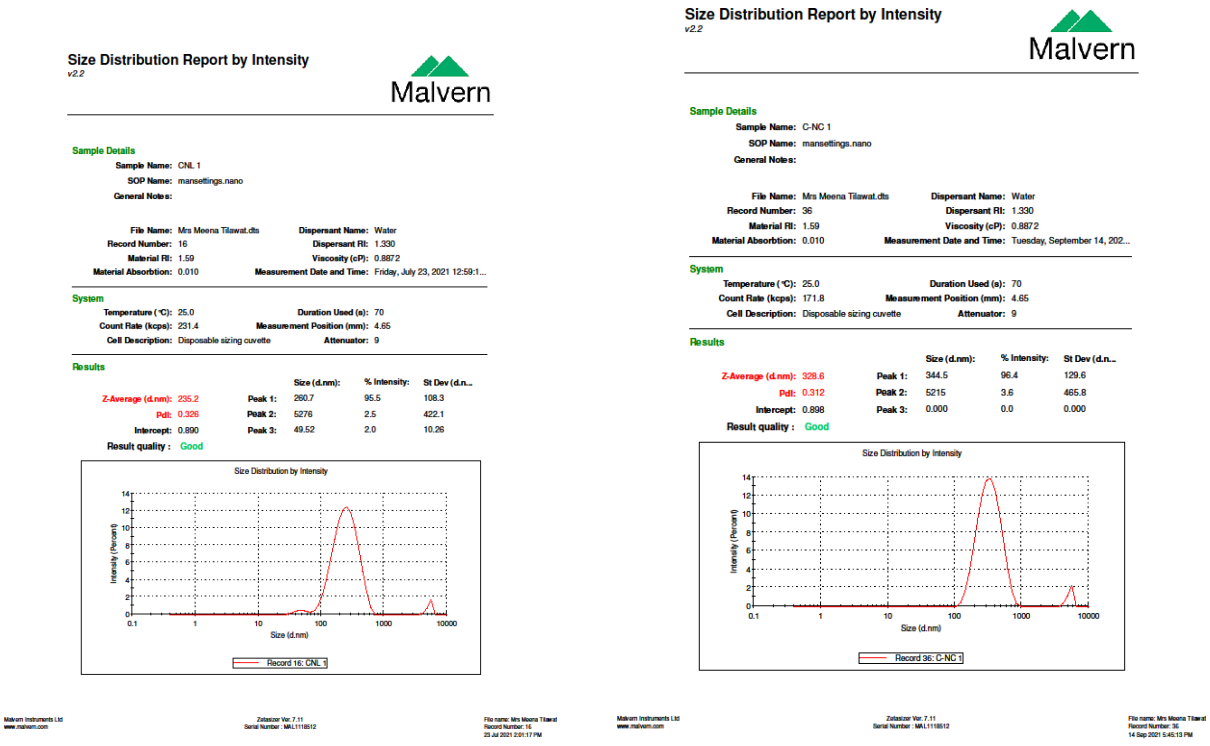


Figure 14: Image showing particle size of curcumin liposomes and curcumin nanocochleates

b) Determination of encapsulation efficiency (EE):

For determination of encapsulation efficiency, 1 mL of formulation transferred in centrifuge tube. Then it has been centrifuged for separation of non-encapsulated curcumin and quercetin at 15000 rpm for 30 minutes. Supernatant and sediment were separated and absolute ethanol added to disrupt and release curcumin and quercetin. It has been further diluted with methanol and absorbances were recorded at 426 nm and 378 nm by UV spectrophotometer. The calibration curve has been plotted and equation noted from calibration curve. Encapsulation efficiency calculated by using formula

$$\% \text{ EE} = \frac{\text{Amount of total drug- amount of free drug}}{\text{Amount of total drug}} \times 100$$

Encapsulation efficiency of curcumin liposomes and curcumin nanocochleates were found to be 75.83% and 82.30 % respectively.

c) Differential scanning calorimetry:

The pure drug, cholesterol, lipid, quercetin-loaded liposomes, and nanocochleates, curcumin loaded liposomes and nanocochleates have been analysed by differential scanning calorimetry (Mettler Toledo DSC1 Star e system Software Mettler STAR e Default DB v.13) to observe thermal behaviour. Figure 15 shows thermographs of pure drug, excipients, liposomes and nanocochleates. The sharp peak of curcumin is observed at 180°C and that of curcumin liposomes and nanocochleates shifted to lower side indicating that curcumin is encapsulated in liposomes and nanocochleates.

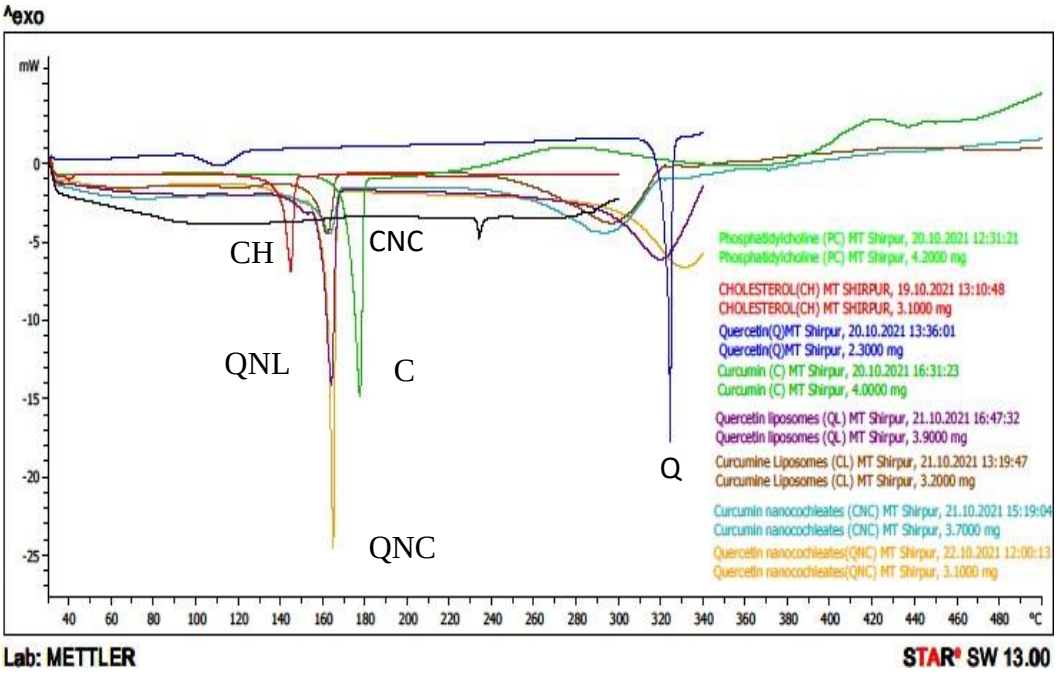

^exo
mW
CH
CNC
QNL
C
Q
QNC
Phosphatidylcholine (PC) MT Shirpur, 20.10.2021 12:31:21
Phosphatidylcholine (PC) MT Shirpur, 4.2000 mg
CHOLESTEROL(CH) MT SHIRPUR, 19.10.2021 13:10:48
CHOLESTEROL(CH) MT SHIRPUR, 3.1000 mg
Quercetin(Q)MT Shirpur, 20.10.2021 13:36:01
Quercetin(Q)MT Shirpur, 2.3000 mg
Curcumin (C) MT Shirpur, 20.10.2021 16:31:23
Curcumin (C) MT Shirpur, 4.0000 mg
Quercetin liposomes (QL) MT Shirpur, 21.10.2021 16:47:32
Quercetin liposomes (QL) MT Shirpur, 3.9000 mg
Curcumine Liposomes (CL) MT Shirpur, 21.10.2021 13:19:47
Curcumine Liposomes (CL) MT Shirpur, 3.2000 mg
Curcumin nanocochleates (CNC) MT Shirpur, 21.10.2021 15:19:04
Curcumin nanocochleates (CNC) MT Shirpur, 3.7000 mg
Quercetin nanocochleates(QNC) MT Shirpur, 22.10.2021 12:00:13
Quercetin nanocochleates(QNC) MT Shirpur, 3.1000 mg
0
-5
-10
-15
-20
-25
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 °C
Lab: METTLER
STAR SW 13.00

Figure 15: **DSC thermograms of pure drug, excipients, liposomes and nanocochleates**

d) Surface morphology:

Surface characteristics of curcumin nanocochleates have been studied by scanning electron microscopy. In the internal structure of nanocochleates, it has been observed as rod shape and the aggregates observed in the image is an indication of trapping method of nanocochleates. Fig.16 confirms the shape of nanocochleates.

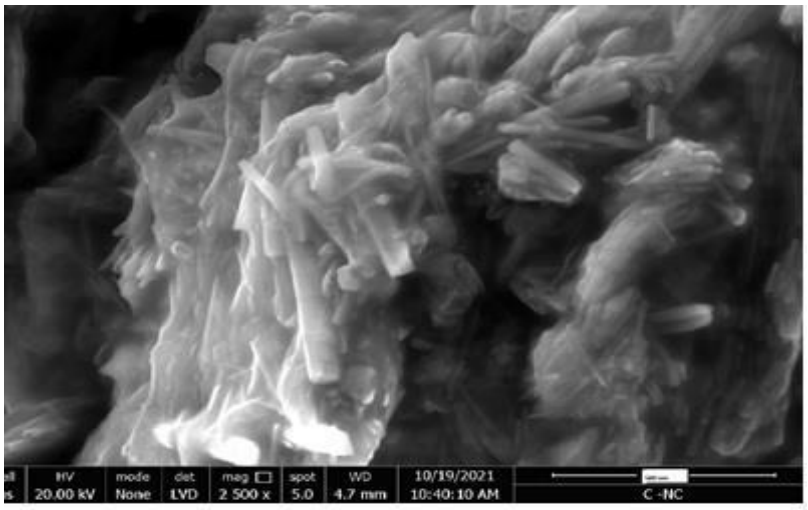


Figure 16: SEM image of curcumin nanocochleates

14 Formulation of nanocochleates gel:

The curcumin and quercetin loaded nanocochleates were further converted to gel formulation by using 1% carbopol (940 gel) under stirring. The formulated gel was evaluated further for various parameters such as pH, spreadability, viscosity etc.

15 Evaluation of gel properties:

a) pH*:* The pH of gel formulation have been analysed by using pH meter (LABINDIA Pico+). pH of gel have been calculated in triplicate and the mean of it is taken as the value. pH of gel was found to be 6.5 which is the pH of carbopol (pH 6-7)

b) Viscosity of gel*:* Viscosity of gel have been evaluated by Brookfield viscometer (DV II Pro+, Brookfield Engineering Lab., Inc., Middleboro. USA). The graph between shear rate and viscosity is plotted.

Viscosity of gel have been found to be in range 750-947 gm/sec. It has been observed that the system is shear-thinning system. The graph of shear rate verses viscosity has been plotted and it is shown in Fig.17

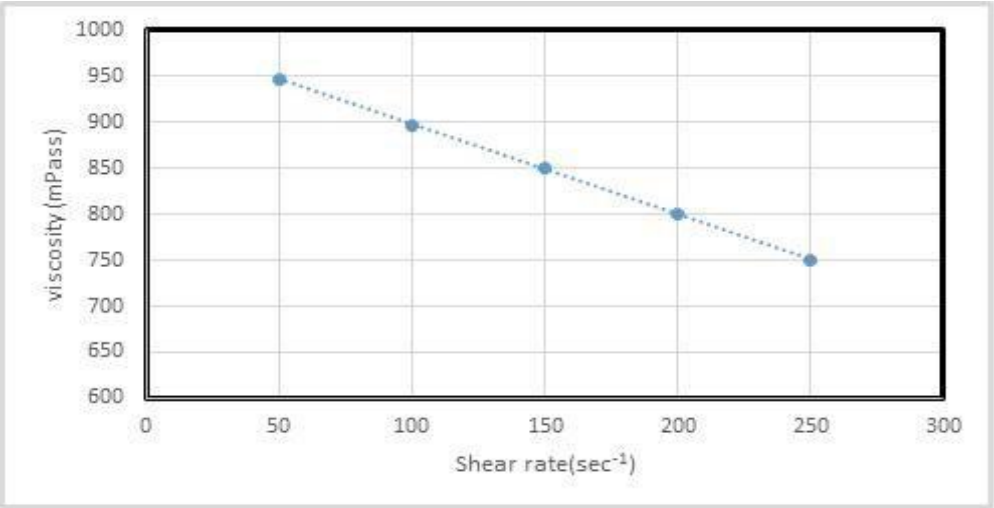


Figure 17: Plot of shear rate verses viscosity of gel

c) Spredability of Gel:

Spreadability of formulations was determined by an apparatus suggested by multimer, which was fabricated itself in laboratory and used for slide fixed on wooded block and upper slide with one end tide to glass slide and other end tied to weight pan. A nanocochleates gel sample of 1 gm was applied between two slides, and a weight of 100 gm was placed on the slides for 5 min to compress the sample into a uniform layer. Spreadability was calculated by the time required to separate the two glass slides.

The formula for calculating spreadability is S= ml/t. Where S= spreadability in gm.cm/sec, l-length of a glass slide, m=110 weight tied to upper slide, t=time in seconds. Glass slide of length 8.3 was used and weight to upper slide 105 gram used throughout the experiment.

Spreadability for prepared gel was found to be 13.12 g.cm/sec and 12.2 gm.cm/sec for marketed formulation

d) *In vitro* drug release*: In vitro* drug release studies have been performed for predicting behaviour of drug *in vitro.* Franz diffusion cell technique is used for analysing *in vitro* release. This study have been carried out on pure drug, quercetin-curcumin loaded nanocochleates and quercetin and curcumin loaded nanocochleates gel. The comparison of *in vitro* drug release of pure drug quercetin, curcumin, quercetin-curcumin loaded nanocochleates and quercetin-curcumin loaded nanocochleates gel have been shown in fig.18

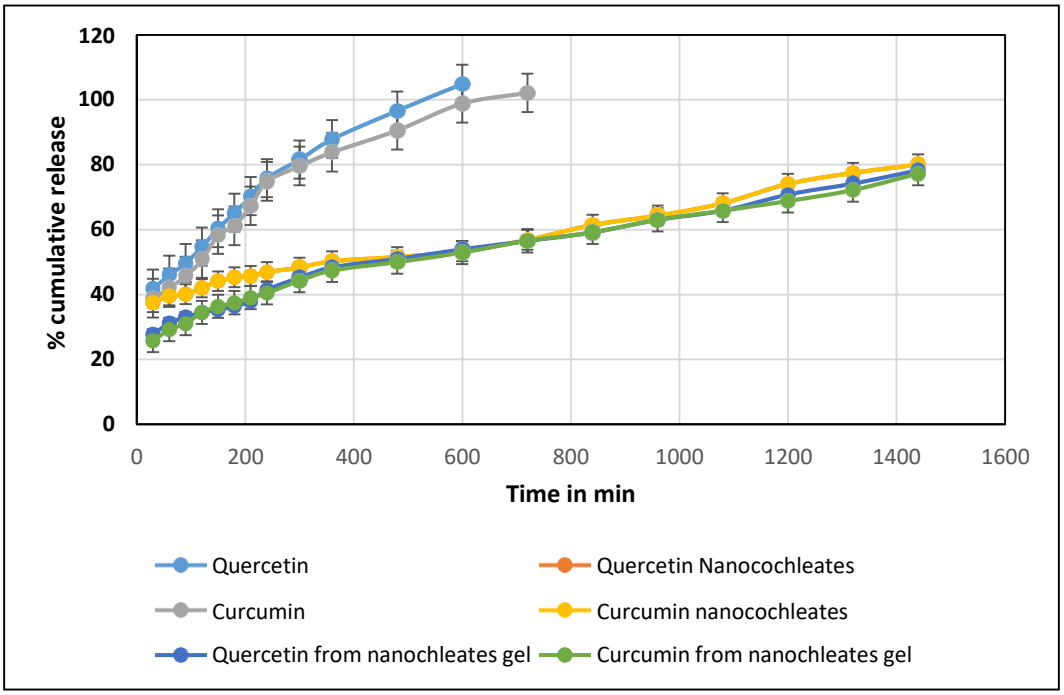


Figure 18: *In vitro* release behaviour of quercetin, quercetin from nanocochleates, quercetin from nanocochleates gel

The first light blue graph indicated drug release of pure drug for period of 24 hours. It has been observed that upto 5 hours almost 90% drug release observed. In case of Quercetin nanocochleates the drug release retarded and observed to be in controlled fashion. Around 80% drug release observed upto 24 hours. In case of curcumin and quercetin loaded nanocochleates, the drug release of quercetin as well as curcumin was found be in controlled fashion. In case of quercetin release from nanocochleates gel, the release not significantly altered but it has been retarded further. It has been observed that 78.19% quercetin has been release from nanocochleates gel upto 24 hrs. In case of curcumin, it has been observed 77.19% drug release from nanocochleates gel. The release pattern was evaluated for various models and observed that kosmeyer peppas model fit for the drug release pattern indicating diffusion mechanism in drug release.

e) *In vitro* anticancer studies:

MTT assay was performed at Deshpande Laboratories, Bhopal using the standard operating procedures. MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] is a pale yellow coloured substance that is cleaved by living cells to yield a dark blue formazan product. This blue formazan formation requires active mitochondria. Hence amount of MTT cleaved by active mitochondria is directly proportional to living viable cells which is further analysed by

colorimetric methods. All compounds were dissolved in dimethyl sulfoxide (DMSO) and further dilutions are made to have a range of test concentration. The concentration of DMSO was <0.1% in all the samples. MCF7 stored at appropriate conditions were used and seeded in 96 well plates. The plates were further treated with various concentrations of test samples. The plates were then incubated at 37°C, 5% CO2 for 96 hours. Further, MTT reagent was added to the wells and then it was incubated for 4 hours. The formed dark blue formazan product was further dissolved in DMSO using safety cabinet and analysed at 550 nm. The percentage inhibition by samples were calculated. The graph between percentage inhibition and concentration were also plotted for calculation of IC50 values.

The IC50 values for quercetin, quercetin liposomes, quercetin nanocochleates, quercetin and curcumin loaded nanocochleates, Untreated samples have been calculated. (Table 15) The graph of concentration verses % inhibition also plotted. (Fig.19)

Table 15: Concentration and related IC 50 values

Concentration	Q	QNL	QNC	QNC-CNC
10	53.64	56.62	61.25	69.85
1	41.28	45.55	47.85	51.26
0.1	13.26	21.65	14.51	38.64
0.01	8.75	4.58	6.98	18.51
0.001	2.59	3.15	2.57	14.57
IC50	3	2	1	0.7

From table 15, it has been observed that quercetin at concentration of 10 micrograms/ml it has 53.64% inhibition on cancer cells, quercetin liposomes has 56.62% inhibition at this concentration, quercetin nanocochleates have more inhibition of cancer cells as compared to quercetin liposomes and found to be 61.25% inhibition. When curcumin has been combined with quercetin in nanocochleates we observed synergistic effect on inhibiting cancerous cells and hence the anticancer action is improved i.e 69.85%inhibition is observed. The IC 50 values for quercetin, quercetin liposomes, quercetin nanocochleates, curcumin and quercetin loaded

nanocochleates were found to be 3, 2, 1, and 0.7 microgram/ml respectively. Hence it has been proved that curcumin and quercetin loaded nanocochleates require least concentration for 50% inhibition in cancerous cells.

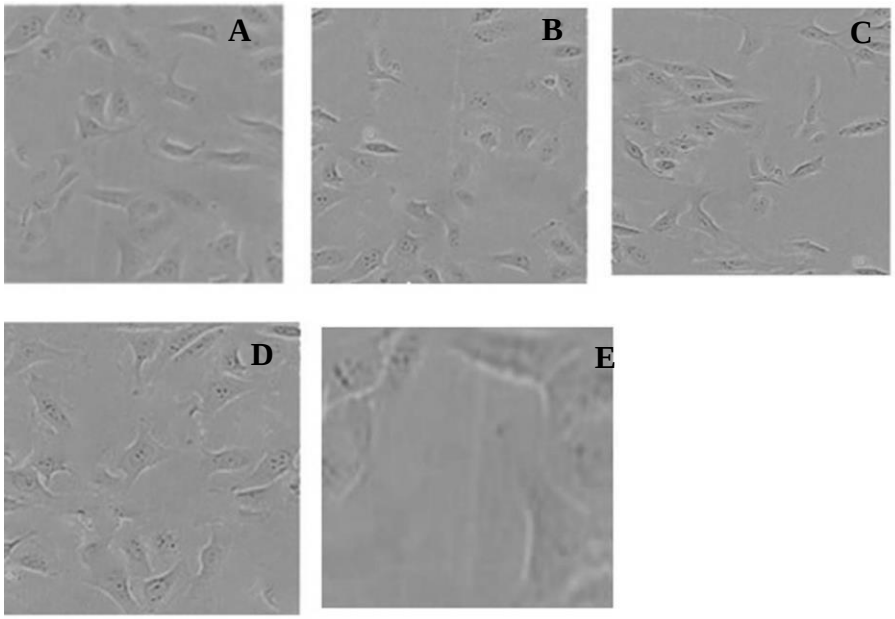


Figure 19: **Image showing effects of control(A), quercetin(C), quercetin liposomes(B), quercetin nanocochleates (D), curcumin and quercetin loaded nanocochleates (E) on cancer cells.**

From fig.19, it has been observed that cancerous cells remain intact, whereas when we treat cancerous cells with quercetin liposomes, the viability of cancerous cells decreased. In comparison to quercetin liposomes the activity further enhanced with quercetin nanocochleates. Hence, it is proven that nanocochleates were efficacious as compared to liposomes for cancer therapy. The anticancer activity found to be excellent when cancer cells were treated with quercetin and curcumin nanocochleates. From literature also the synergism had already proven for quercetin and curcumin [37-41]. Thus this novel delivery serves a better option for delivering curcumin and quercetin in combination for cancer therapy.

f) *Ex vivo* skin permeation studies:

Ex-vivo skin permeation studies have been performed by using goat skin. The ear pinna skin have been used since it is waste product of slaughter house. The hairs on ear pinna skin have been excised and skin was first washed with water and then 0.1 N normal saline solution. About 2 cm part of skin was used for study. The *ex–vivo* skin permeation have been performed on

nanocochleates gel and marketed gel formulation for 24 hours. Aliquots have been withdrawn at specific time intervals and analysed by HPLC. The concentration of drug have been calculated by calibration curve [42,43]. The skin permeation and flux was calculated from the formula mentioned in literature [44,45]. The formula is as follows

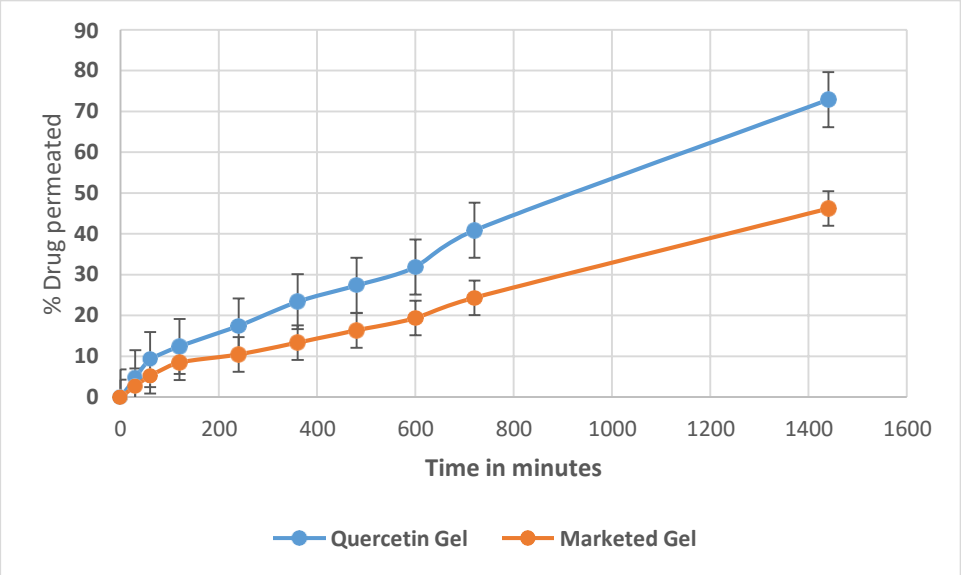


Figure 20: *Ex vivo* skin permeation of quercetin from nanocochleates gel and marketed quercetin gel formulation

$P = J / C_O$

where p=permeability coefficient

J=flux

C_O= Initial concentration

Ex-vivo skin permeation of quercetin from nanocochleates gel and marketed gel is shown in fig.20

Table 16: Permeation parameters for quercetin release for nanocochleates gel and quercetin marketed gel

Formulation	Flux	permeability coefficient
Nanocochleates gel	20.125 microgram/cm2 h	0.1
Marketed gel	8.61 microgram/cm2 h	0.004

The amount of drug permeated from nanocochleates gel was found to be 72.86% and for marketed gel formulation, it was found to be 46.19% over period of 24 hrs. It has shown that nanocochleates gel shown better skin permeation may be because the lipid delivery has ease for penetration and avoids barrier for skin permeation. (Fig.20). The flux for nanocochleates gel found to be 20.125 microgram/cm2 h and for marketed gel formulation is 8.61 microgram/cm2 h. The permeability coefficient for nanocochleates gel and Marketed gel were found to be 0.1 and 0.04 respectively (Table 16).

g) Stability studies:

The shelf life of the product is determined by stability studies, which ensure the formulation remains within its predefined limits over a prescribed time. Therefore, the stability studies of gel was carried out after storing them at different temperature and relative humidity conditions for three months as per ICH guidelines [46]. The formulation have been withdrawn initially for 15 days and after wards for every month and were evaluated for changes in pH, spredability and percent drug content. pH have been analysed by pH meter. Spredability have been performed by apparatus suggested by multimer. Drug content have been calculated by developed HPLC method. The results were tabulated as below (Table 17). It has been observed that no significant difference have been found out in pH, spredability and drug content indicating a stable formulation.

Table 17: Stability studies of gel for 3 months and corresponding pH, spredability and drug content

Time points(in months)	**pH**	**Spreadability**	**Drug content**
Temperature 25± 2 C/60±5%RH			
Initial	6.2	13.12	100
0.5	6.1	13.2	101
1	6.3	12.98	99.4
2	6.3	12.58	98.5
3	5.9	13.25	99.2
Temperature 5°C ± 3°C			
Initial	6.2	13.12	99.6
0.5	6.1	13.25	98.5
1	5.9	12.98	98.9
2	6.2	12.92	99.9
3	6.4	13.02	98.8

h) Drug release from nanocochleate gel after stability studies:

In vitro drug release studies have been performed for predicting behaviour of drug *in vitro.* Franz diffusion cell technique is used for analysing *in vitro* release. After stability studies the drug release for quercetin from and curcumin from same nanocochleates gel sample were found to be 77.39±0.15% and 76.58±0.45%. No significant difference in drug release were observed. Hence the formulation is found to be stable.

16 Expected outcome:

In women, the Breast cancer is common cause of mortality. So this present work will be helpful for the women in improving the breast cancer treatment. The nanocochleates gel also helpful for avoiding the drug related untoward effects by producing targeted action which are associated with available conventional treatment. Overall, the well-being of a women can be improved, and thereby there is a better treatment option for breast cancer in society. If the Breast cancer patients will get the better therapy, there will be definitely improvement in the society regarding awareness of the disease and the therapy options for the disease.

Publication details:

Article name	Name of Journal	Status	Impact factor
1) Nanocochleates: A Potential drug delivery system	Journal of Molecular liquids	Published	6.16
2) Research article on formulation and evaluation	Particulate science and technology	Published	2.35
3) Research article on formulation	Journal of drug delivery science and technology	submitted	5.06
3) Research article on Simultaneous estimation method by HPLC	Iranian Journal of Science and Technology	Under review	1.5

Key References:

1. Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and mortality and epidemiology of breast cancer in the world. Asian Pac J Cancer Prev. 2016;17(S3):43-6.
2. Shrestha H, Bala R, Arora S. Lipid-based drug delivery systems. Journal of pharmaceutics. 2014;2014.
3. Chime SA, Onyishi IV. Lipid-based drug delivery systems (LDDS): Recent advances and applications of lipids in drug delivery. Afr. J. Pharm. Pharmacol. 2013 Dec 29;7(48):3034-59.
4. L Arias J, Clares B, E Morales M, Gallardo V, A Ruiz M. Lipid-based drug delivery systems for cancer treatment. Current drug targets. 2011 Jul 1;12(8):1151-65.
5. Alavi M, Karimi N, Safaei M. Application of various types of liposomes in drug delivery systems. Advanced pharmaceutical bulletin. 2017 Apr;7(1):3.
6. Guimarães D, Cavaco-Paulo A, Nogueira E. Design of liposomes as drug delivery system for therapeutic applications. International journal of pharmaceutics. 2021 May 15;601:120571.
7. Ahiwale, R., B. Chellampillai, and A. Pawar. 2020. Investigation of 1, 2-dimyristoyl-Sn-glycero-3-phosphoglycerol-sodium (DMPG-Na) Lipid with various metal cations in nanocochleate preformulation: Application for Andrographolide oral delivery in cancer therapy. AAPS PharmSciTech 21 (7):1–8. doi: 10.1208/s12249-020-01801-1.
8. Ahiwale, R., B. Chellampillai, and A. Pawar. 2022. Investigation of novel sorafenib tosylate loaded biomaterial based nano-cochleates dispersion system for treatment of hepatocellular carcinoma. Journal of Dispersion Science and Technology 43 (10):1568–86. doi: 10.1080/01932691.2021.1878034
9. Pawar A, Bothiraja C, Shaikh K, Mali A. An insight into cochleates, a potential drug delivery system. RSC advances. 2015;5(99):81188-202.
10. Bothiraja C, Yojana BD, Pawar AP, Shaikh KS, Thorat UH. Fisetin-loaded nanocochleates: formulation, characterisation, in vitro anticancer testing, bioavailability and biodistribution study. Expert opinion on drug delivery. 2014 Jan 1;11(1):17-29.
11. Bothiraja C, Rajput N, Poudel I, Rajalakshmi S, Panda B, Pawar A. Development of novel biofunctionalized chitosan decorated nanocochleates as a cancer targeted drug delivery platform. Artificial cells, nanomedicine, and biotechnology. 2018 Oct 31;46(sup1):447-61.
12. Poudel I, Ahiwale R, Pawar A, Mahadik K, Bothiraja C. Development of novel biotinylated chitosan-decorated docetaxel-loaded nanocochleates for breast cancer

targeting. Artificial cells, nanomedicine, and biotechnology. 2018 Nov 5;46(sup2):229-40.

13. Asprea M, Tatini F, Piazzini V, Rossi F, Bergonzi MC, Bilia AR. Stable, monodisperse, and highly cell-permeating nanocochleates from natural soy lecithin liposomes. Pharmaceutics. 2019 Jan 16;11(1):34.
14. Liu M, Zhong X, Yang Z. Chitosan functionalized nanocochleates for enhanced oral absorption of cyclosporine A. Scientific reports. 2017 Jan 23;7(1):1-0.
15. Zhong X, Chen B, Yang Z. Nanocochleates as the potential delivery systems for oral antitumor of hydroxycamptothecin. Journal of Biomedical Nanotechnology. 2018 Jul 1;14(7):1339-46.
16. Çoban Ö, Değim Z, Yılmaz Ş, Altıntaş L, Arsoy T, Sözmen M. Efficacy of targeted liposomes and nanocochleates containing imatinib plus dexketoprofen against fibrosarcoma. Drug development research. 2019 Aug;80(5):556-65.
17. Pham TT, Barratt G, Michel JP, Loiseau PM, Saint-Pierre-Chazalet M. Interactions of antileishmanial drugs with monolayers of lipids used in the development of amphotericin B–miltefosine-loaded nanocochleates. Colloids and Surfaces B: Biointerfaces. 2013 Jun 1;106:224-33.
18. Tamargo B, Monzote L, Piñón A, Machín L, García M, Scull R, Setzer WN. In vitro and in vivo evaluation of essential oil from Artemisia absinthium L. formulated in nanocochleates against cutaneous leishmaniasis. Medicines. 2017 Jun 9;4(2):38.
19. Machín L, Tamargo B, Piñón A, Atíes RC, Scull R, Setzer WN, Monzote L. Bixa orellana L.(Bixaceae) and Dysphania ambrosioides (L.) Mosyakin & Clemants (Amaranthaceae) essential oils formulated in nanocochleates against Leishmania amazonensis. Molecules. 2019 Nov 20;24(23):4222.
20. Castro AL, Pomel S, Cailleau C, Fournier N, Dennemont I, Loiseau PM, Barratt G. Pharmacokinetics, biodistribution, and activity of amphotericin B-loaded nanocochleates on the Leishmania donovani murine visceral leishmaniasis model. International Journal of Pharmaceutics. 2022 Aug 25;624:121985.
21. Shanmugam T, Joshi N, Ahamad N, Deshmukh A, Banerjee R. Enhanced absorption, and efficacy of oral self-assembled paclitaxel nanocochleates in multi-drug resistant colon cancer. International Journal of Pharmaceutics. 2020 Aug 30;586:119482.
22. Yücel Ç, Karatoprak GŞ, Atmar A. Novel resveratrol-loaded nanocochleates and effectiveness in the treatment of diabetes. Fabad Journal of Pharmaceutical Sciences. 2018 Apr 1;43(2):125-34.

23. Shanmugam T, Joshi N, Kaviratna A, Ahamad N, Bhatia E, Banerjee R. Aerosol delivery of paclitaxel-containing self-assembled nanocochleates for treating pulmonary metastasis: An approach supporting pulmonary mechanics. ACS Biomaterials Science & Engineering. 2020 Dec 21;7(1):144-56.
24. Srivastava NS, Srivastava R (2019) Curcumin and quercetin synergistically inhibit cancer cell proliferation in multiple cancer cells and modulate Wnt/β-catenin signaling and apoptotic pathways in A375 cells. Phytomedicine. 52:117-28. DOI: 10.1016/j.phymed.2018.09.224
25. https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin
26. https://pubchem.ncbi.nlm.nih.gov/compound/Curcumin
27. Chidambaram M, Krishnasamy K. Drug-drug/drug-excipient compatibility studies on curcumin using non-thermal methods. Advanced pharmaceutical bulletin. 2014;4(3):309.
28. ICH (2005) ICH Topic Q2 (R1) Validation of analytical procedures text and methodology. Int Conf Harmon 1994:17. https ://www.ich.org/file admin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_G uide line.pdf
29. Samad A, Sultana Y, Aqil M. Liposomal drug delivery systems: an update review. Current drug delivery. 2007 Oct 1;4(4):297-305.
30. Shaker S, Gardouh AR, Ghorab MM. Factors affecting liposomes particle size prepared by ethanol injection method. Research in pharmaceutical sciences. 2017 Oct;12(5):346.
31. Ferreira SC, Bruns RE, Ferreira HS, Matos GD, David JM, Brandão GC, da Silva EP, Portugal LA, Dos Reis PS, Souza AS, Dos Santos WN. Box-Behnken design: An alternative for the optimization of analytical methods. Analytica chimica acta. 2007 Aug 10;597(2):179-86.
32. Gaikwad VL, Choudhari PB, Bhatia NM, Bhatia MS. Characterization of pharmaceutical nanocarriers: In vitro and in vivo studies. InNanomaterials for drug delivery and therapy 2019 Jan 1 (pp. 33-58). William Andrew Publishing.
33. D'Souza S. A Review ofIn VitroDrug Release Test Methods for Nano-Sized Dosage Forms. Advances in pharmaceutics. 2014;2014:1-2.
34. Gill P, Moghadam TT, Ranjbar B. Differential scanning calorimetry techniques: applications in biology and nanoscience. Journal of biomolecular techniques: JBT. 2010 Dec;21(4):167.

35. Yaneva Z, Georgieva N. Physicochemical and morphological characterization of pharmaceutical nanocarriers and mathematical modeling of drug encapsulation/release mass transfer processes. InNanoscale fabrication, optimization, scale-up and biological aspects of pharmaceutical nanotechnology 2018 Jan 1 (pp. 173-218). William Andrew Publishing.
36. Chen L, Yue B, Liu Z, Luo Y, Ni L, Shen W, Zhou Z, Ge X. Preparation of curcumin-loaded cochleates: characterisation, stability and antioxidant properties. Food & Function. 2022;13(21):11273-82.
37. Nadaf SJ, Killedar SG. Curcumin nanocochleates: Use of design of experiments, solid state characterization, in vitro apoptosis and cytotoxicity against breast cancer MCF-7 cells. Journal of Drug Delivery Science and Technology. 2018 Oct 1;47:337-50.
38. S. Kundur, A. Prayag, P. Selvakumar, Nguyen H, McKee L, Cruz C, Srinivasan A, Shoyele S, Lakshmikuttyamma A. Synergistic anticancer action of quercetin and curcumin against triple-negative breast cancer cell lines. J. Cell. Physiol. 234(2019) 11103-111018. https://doi.org/10.1002/jcp.27761
39. F. Mansourizadeh, D. Alberti, V. Bitonto, M. Tripepi, H. Sepehri, S. Khoe Crich. Efficient synergistic combination effect of Quercetin with Curcumin on breast cancer cell apoptosis through their loading into Apo ferritin cavity. Colloids Surf B Biointerfaces. 191(2020) 110982. https://doi.org/10.1016/j.colsurfb.2020.110982
40. V. Sharma, L. Kumar, S.K. Mohanty, J.P. Maikhuri, S. Rajender, G. Gupta Sensitization of androgen refractory prostate cancer cells to anti-androgens through re-expression of epigenetically repressed androgen receptor–synergistic action of quercetin and curcumin. Mol. Cell. Endocrinol. 431(2016) 12-https://doi.org/10.1016/j.mce.2016.04.024.
41. A. Bahuguna, I .Khan, V.K. Bajpai, S.C. Kang. MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh J. Pharmacol. 12(2017) 115-118
42. V. Gupta, P.Trivedi. Ex vivo localization and permeation of cisplatin from novel topical formulations through excised pig, goat, and mice skin and in vitro characterization for effective management of skin-cited malignancies. Artif Cells Nanomed Biotechnol 43(2015) 373-382. https://doi.org/10.3109/21691401.2014.893523
43. N.Ahmad, R. Ahmad, T.M. Buheazaha, H.S. AlHomoud, H.A. Al-Nasif, M.Sarafroz. A comparative ex vivo permeation evaluation of a novel 5-Fluorocuracil nanoemulsion-gel by topically applied in the different excised rat, goat, and cow skin. Saudi J. Biol. Sci. 27(2020) 1024-1040. https://doi.org/10.1016/j.sjbs.2020.02.014

44. N. Dudhipala, R. Phasha Mohammed, A. Adel Ali Youssef, N. Banala. Effect of lipid and edge activator concentration on development of aceclofenac-loaded transfersomes gel for transdermal application: In vitro and ex vivo skin permeation. Drug Dev. Ind. Pharm. 46(2020) 1334-1344.https://doi.org/10.1080/03639045.2020.1788069
45. S. Ahmed, M.A. Kassem, S. Sayed. Bilosomes as promising nanovesicular carriers for improved transdermal delivery: construction, in vitro optimization, ex vivo permeation and in vivo evaluation. Int. J. Nanomed. 15 (2020) 9783- 9798 doi: 10.2147/IJN.S278688
46. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use ICH harmonized tripartite guideline stability testing of new drug substances and products Q1A(R2)

Ph.D. student

Mrs. Meena Tilawat

M.Pharm

Supervisor

Dr.Smita Bonde

Associate Professor

Quality assurance